dermis of mutant mice are B220(+)CD3(-)CD19(-) plasmacytoid dendritic cells and (C) express both IFNα and IL-6. For panels B-C: Isotype control-treated: n = 5 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$); β1aAb-treated: n = 4 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$). For panel E: n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). DE = D1545E. WC = W1572C. * $p<0.05$, ** $p<0.01$, † $p<0.001$, ‡ $p<0.0001$.

Supplementary Figure Legends

Figure S1. (A) Schematic of constructs used to generate *Fbn1*$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice by homologous recombination. The construct contained a neomycin resistance cassette (NeoR), flanked by loxP sites, that was later removed via breeding to mice expressing Cre-recombinase. (B) Representative Southern blot (for mutation W1572C) showing proper targeting in embryonic stem (ES) cells prior to blastocyst injection and implantation into pseudopregnant mice. (C) Mice were genotyped on the basis of creation of a new AciI site (W1572C) or destruction of a BsmAI site (D1545E) in correctly targeted mice. *Fbn1* genotypes: WC/+, *Fbn1*$^{DW1572C/+}$; DE/+, (*Fbn1*$^{D1545E/+}$).

Figure S2. (A) Masson's trichrome staining of back skin sections from mutant (genotypes indicated) female mice at 1 month (top panels) and 3 months (bottom panels) of age demonstrates progressive loss of subcutaneous fat and an expanded zone of dense dermal collagen. (B) Quantification of the thickness of the zones of dermal collagen a nd subcutaneous fat in wild-type and mutant female mice at 1 (top panels) and 3 (bottom panels) months of age. 1 month

females: n = 8 (+/+), 8 (WC/+), 8 (WC/WC), 9 (DE/+); 3 month females: n = 12 (+/+), 10 (WC/+), 9 (WC/WC), 9 (DE/+). Scale bars, 50 μm. * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001. (C) Electron microscopy shows excessive microfibrillar deposits (black arrows) and sparsely distributed electron-dense (black) elastin in mutant skin. Scale bars, 500 nm.

Figure S3. Flow cytometry analysis showing that cell-surface expression of total αvβ3 or αvβ5 were normal in SSS mice and did not change with β1aAb treatment.

Figure S4. (A) Schematic showing how the stretched skin area/total surface area (SSA/TSA) ratios were measured. Mice were anesthetized and their back-hair removed. Mice were then briefly suspended by their back skin and photographed in profile in a uniform manner. (B) Mutant mice showed a reduced SSA/TSA ratio that was normalized upon treatment with β1aAb but not by an isotype-matched control (IgG). (C) There were no differences in body weight between all experimental groups. Isotype control-treated: n = 12 (*Fbn1*$^{+/+}$), 9 (*Fbn1*$^{D1545E/+}$), 8 (*Fbn1*$^{W1572C/+}$); β1aAb-treated: n = 12 (*Fbn1*$^{+/+}$), 10 (*Fbn1*$^{D1545E/+}$), 10 (*Fbn1*$^{W1572C/+}$). Measurements were performed with NIH image J software (National Institute of Health, Bethesda, MD, USA). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S5. (A) Introduction of haploinsufficiency (-/+) or the null state (-/-) for the gene encoding integrin β3 (*Itgb3*) attenuates or prevents skin stiffening, respectively, in mouse models of SSS. (B) Histologic and morphometric analyses using Masson's trichrome stain. (C) A small subset of mice (~12% overall) that are haploinsufficient (-/+) or null (-/-) for *Itgb3*, the gene encoding integrin β3, show focal epidermal hyperplasia and increased cellularity and collagen in the dermis at five months of age. These findings were observed irrespective of *Fbn1* genotype. Scale bars, 50 μg. n = 12 (*Fbn1*$^{+/+}$ and *Itgb3*$^{+/+}$), 13 (*Fbn1*$^{+/+}$ and *Itgb3*$^{-/+}$), 7 (*Fbn1*$^{+/+}$ and *Itgb3*$^{-/-}$), 8 (*Fbn1*$^{D1545E/+}$ and *Itgb3*$^{+/+}$), 18 (*Fbn1*$^{D1545E/+}$ and *Itgb3*$^{-/+}$), 14 (*Fbn1*$^{D1545E/+}$ and *Itgb3*$^{-/-}$), 7 (*Fbn1*$^{W1572C/+}$ and *Itgb3*$^{+/+}$), 9 (*Fbn1*$^{W1572C/+}$ and *Itgb3*$^{-/+}$), 6 (*Fbn1*$^{W1572C/+}$ and *Itgb3*$^{-/-}$). Scale bars, 50 μc. * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S6. (A) Flow cytometry analysis did not reveal an increase in the expression of integrins known to potently support the activation of TGFβ (αvβ5, αvβ6 or αvβ8) in the dermis of mutant mice, when compared to wild-type littermates. (B) Immunofluorescence analysis reveals increased latency-associated peptide (LAP)-1, LAP-2 and total TGFβ2 in the dermis of mutant mice, when compared to wild-type littermates. No difference in active (free) TGFβ1 was observed. n = 5 (*Fbn1*$^{+/+}$, +/+), 4 (*Fbn1*$^{D1545E/+}$, DE/+), 4 (*Fbn1*$^{DW1572C/+}$, WC/+). Scale bars, 50 μm.

Figure S7. Increased circulating levels of anti-nuclear and anti-topoisomerase I antibodies by enzyme-linked immunosorbent assay (ELISA) in mutant mice at 18 months of age. n = 5 (*Fbn1*$^{+/+}$, +/+), 4 (*Fbn1*$^{D1545E/+}$, DE/+), 4 (*Fbn1*$^{DW1572C/+}$, WC/+). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S8. (A) Increased deep dermal expression of active integrin β3 in the mutant skin colocalizes with CD45(+) cells derived from the bone marrow; both signals were normalized upon treatment with β1aAb but not with an isotype-matched control (IgG). Isotype control-treated: n = 5 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$); β1aAb-treated: n = 4 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$). For panel E: n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001. (B) Gating strategy for pDC identification: First, live cells are gated. Next, CD11b(+) leukocytes, CD3(+) T cells, and CD19(+) B cells are excluded. (C) Representative flow cytometry plots showing that the cells under consideration are B220+, CD317(high), Siglec H(+), Ly6C(high) and show a conventional size distribution for pDCs. (D) Immunofluorescent staining confirming the presence of Siglec H(+) cells in the dermis of placebo-treated SSS mice, but absent in that of of wild-type or treated animals. Scale bars, 50 μm. Isotype control-treated: n = 5 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$); β1aAb-treated: n = 4 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$). For panel E: n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001. (E) CD11b(-)CD3(-)CD19(-)CD317(high) cells are expressing interferon α, as expected for activated pDCs, as well as interleukin-6. Percentages shown indicate % of total dermal cells. n = 5 (*Fbn1*$^{+/+}$, +/+), 4

(*Fbn1*$^{D1545E/+}$, DE/+), 4 (*Fbn1*$^{DW1572C/+}$, WC/+). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S9. (A) The skewing of T helper (Th) CD4(+) lymphocytes toward IL-4(+) Th2 and IL-17(+) Th17 populations in mutant mice was prevented upon treatment with β1aAb. Isotype control-treated: n = 5 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$); β1aAb-treated: n = 4 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$). For panel E: n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001. (B) Representative flow cytometry plots of dermal cells positive for CD3 and interleukins 17, 9, 22, 4, and 13. (C) Quantification by boxplot shows increases in CD3(+) cells also positive for interleukins 17, 9, 22, 4, and 13, and in CD3(-) cells also positive for interleukins-9 or interleukin-22. (D) There were no changes in either FoxP3(+) CD4(+)T-regulatory (Treg) or IFNγ(+) CD4(+)Th1 cells in the dermis of SSS mice. All mice were male and 2 months of age. n = 5 (*Fbn1*$^{+/+}$, +/+), 4 (*Fbn1*$^{D1545E/+}$, DE/+), 4 (*Fbn1*$^{DW1572C/+}$, WC/+). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S10. Mutant mice showed accumulation of B220(high)CD19(+) activated B cells and CD138(+)B220(low)CD19(+) plasma cells in the dermis that was prevented by treatment with β1aAb but not an isotype-matched control (IgG). Isotype control-treated: n = 5 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$); β1aAb-treated: n = 4 (*Fbn1*$^{+/+}$), 7 (*Fbn1*$^{D1545E/+}$). For panel E: n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S11. (A) TGF-β neutralizing antibody (TGFβNAb) reverses accumulation of pDCs (defined by B220(+)CD3(-)CD19(-)) in the dermis of *Fbn1*$^{D1545E/+}$ mice, and (B) the expression of both IFNα and IL-6 in these cells. Both (C) the skewing of T helper (Th) CD4(+) lymphocytes toward IL-4(+) Th2 and IL-17(+) Th17 populations, and (D) the accumulation of B220(high)CD19(+) activated B cells and CD138(+)B220(low)CD19(+) plasma cells in the dermis of *Fbn1*$^{D1545E/+}$ mice were reversed upon treatment with TGFβNab, but not an isotype-matched control (IgG). Isotype control-treated: n = 4 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$). TGFβNAb-treated: n = 4 (*Fbn1*$^{+/+}$); 4 (*Fbn1*$^{D1545E/+}$). * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S12. Adherence and activation of plasmacytoid dendritic cells (pDCs) *in vitro*. (A) Wild-type spleen-derived pDCs show an increase in adherence to the matrix elaborated by murine embryonic fibroblasts (MEFs) derived from *Fbn1*$^{W1572C/+}$ (WC/+, n = 8) and *Fbn1*$^{W1572C/W1572C}$ (WC/WC, n = 4) SSS mice, when compared to *Fbn1*$^{+/+}$ (+/+, n = 6) mice. (B) Among adherent pDCs, those plated on mutant MEFs show increased expression of WOW-1, integrin α5β1, IL-6, and IFNα. * p<0.05, ** p<0.01, † p<0.001, ‡ p<0.0001.

Figure S13. Cultured SSc dermal fibroblasts show increased total β1 integrin by flow cytometry. Treatment with β3 integrin-blocking antibody (β3bAb) did not significantly reduce cell-surface presentation of total β1 integrin. Quantifications reflect the analysis of 6 control and 5 SSc cell lines. * p<0.05.

Figure S14. Expression and signaling abnormalities in SSc fibroblasts are attenuated by integrin-modulating antibodies. (A) Cultured primary SSc fibroblasts show high surface expression of WOW-1 that was normalized by treatment with β1aAb. Representative flow cytometry histograms depict the percent of maximum (y-axis) at various fluorescent intensities (x-axis), as described in Supplemental Methods. Quantification of the percent of positive cells is also shown. Total αvβ3 and αvβ5 were normal in SSc cells and did not change with treatment. (B) Cultured SSc fibroblasts (red) show low expression of microRNA-29a (miR-29a) and high expression of messenger RNAs (mRNAs) derived from the genes encoding types IA2 (COL1A2) and III (COL3A1) collagens, when compared to age- and gender-matched control fibroblasts (green); each of these abnormalities was normalized upon treatment with β1aAb in a dose-dependent manner. (C) SD208, an antagonist of the kinase activity of the type I TGFβ receptor subunit (TβRI), normalizes expression of the genes encoding type IA2 and III collagens in primary dermal fibroblasts derived from patients with SSc. Although treatment increased expression of miR-29a, this finding did not reach significance. (D, E) Control fibroblasts show phosphorylation of Smad3 (pSMAD3) in response to 5 minutes of stimulation with TGFβ1, without a change in phosphorylated extracellular regulated kinase1/2 (pERK1/2). Neither signaling cascade was attenuated by β1aAb, β3bAb or β1 integrin-blocking antibody (β1bAb). In contrast, SSc fibroblasts uniquely show ERK1/2 activation (pERK1/2) in response to TGFβ1 that was normalized after treatment with β1aAb

or β3bAb but not β1bAb. Both Smad3 and ERK1/2 activation were sensitive to treatment with SD208, an antagonist of the kinase activity of the type I TGFβ receptor subunit (TβRI). (F) U0126, an inhibitor of the mitogen-activated protein kinase/ERK kinase (MEK), increases miR-29a expression and reduces type IA2 and III collagen expression in SSc fibroblasts. Quantifications for panels A-F reflect the analysis of 6 control and 5 SSc cell lines. * $p<0.05$, ** $p<0.01$, † $p<0.001$, ‡ $p<0.0001$. (G) Flow cytometry reveals that CD317(+) pDCs show high phosphorylation of ERK1/2 (pERK) in SSS mouse models; n = 5 (*Fbn1*$^{+/+}$), 4 (*Fbn1*$^{D1545E/+}$), 4 (*Fbn1*$^{DW1572C/+}$). (H,I) MEK inhibitor RDEA119 prevents skin stiffness, dermal collagen accumulation and loss of subcutaneous fat in *Fbn1*$^{D1545E/+}$ mice. For panels G-I, Isotype control-treated: n = 11 (*Fbn1*$^{+/+}$), 10 (*Fbn1*$^{D1545E/+}$); RDEA119-treated: n = 10 (*Fbn1*$^{+/+}$), 10 (*Fbn1*$^{D1545E/+}$). * $p<0.05$, ** $p<0.01$, † $p<0.001$, ‡ $p<0.0001$.

Figure S15. Events influencing and influenced by plasmacytoid dendritic cells (pDCs). The abnormal extracellular matrix (ECM) in SSS leads to concentration of TGFβ in the skin. TGFβ can induce expression of itself and IL-6 by pDCs; the combination of TGFβ and IL-6 leads to Th17 skewing. pDCs also secrete type I interferon (IFN-α/β), which together with IL-6 can induce Th1 polarization and the activation/maturation of plasma cells and autoreactive B cells. IFN-α/β can also induce myeloid dendritic cells (mDCs) to phagocytize cellular debris, which can indirectly contribute to autoantibody production (dashed arrow). pDCs can also contribute to Th2 polarization through secretion

of OX40L or IL-4 and the Th2 cytokines IL-4 and IL-13 can influence pDC performance. The expression of integrins by pre-pDCs, perhaps in response to an altered ECM, can influence their transmigration, adhesion and/or maturation to pDCs.

Figure S16. (A) There were no differences in final blood cell counts between isotype control- and β1aAb-treated animals. n = 3 for each experimental group. Nml Range = the normal values reported by the Comparative Pathology Laboratory at Johns Hopkins University School of Medicine. K/ μL = thousands per cubic microliter of blood. M/ μL = millions per cubic microliter of blood. (B) Specificity of the WOW-1 antibody for integrin αvβ3 in its active conformation was assessed in control fibroblasts by flow cytometry. As expected, chelation of calcium with 10 mM Ethylenediaminetetraacetic acid (EDTA) – an event known to prevent the active conformation of αvβ3 – reduced immunoreactivity, while treatment with 2 mM $MnCl_2$ – known to activate αvβ3 – increased immunoreactivity.

Methods

Subjects

Patients were recruited from the Scleroderma Center and Connective Tissue Clinic at Johns Hopkins Hospital (F.M.W. and H.C.D.). All skin biopsies and

research protocols were performed in compliance with the Johns Hopkins School of Medicine Institutional Review Board and after informed consent.

Mice

All mice were cared for under strict compliance with the Animal Care and Use Committee of the Johns Hopkins University School of Medicine. *Fbn1*$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice were generated by homologous recombination as described in the next section. *Itgb3* +/- mice were purchased through Jackson Laboratories (Bar Harbor, ME) as heterozygotes. All experimental mice were on a mixed C57Bl/6J and 129/SvEv background. To minimize potentially confounding background effects, all comparisons between genotypes and between treatment arms within a genotype were made between sex-matched littermates; no other randomization procedures were applied. Sample size estimates for assessment of therapeutic responses were based upon the penetrance and severity of the specific parameter under consideration in untreated cohorts.

***Generation of Fbn1*$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice**

Fbn1$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice were generated by homologous recombination (Figure S1A). A 10 kb *Fbn1* fragment was generated by PCR from mouse genomic tail DNA, digested with Acc65 and NheI restriction enzymes (NEB), and ligated into pSL301 (Invitrogen Corp.). Site-directed mutagenesis (SDM) was performed using the QuikChange mutagenesis kit (Stratagene Inc.), creating either the D1545E or W1572C mutation. The targeting vector was

assessed by sequence analysis. SDM was again performed to remove the AatII restriction site from pSL301. The NeoR cassette was amplified from pEGFP-C1 (Invitrogen Corp.) and the amplicon was subcloned into pCR2.1-TOPO (Invitrogen Corp.). A fragment containing the AatII restriction site and NeoR, with flanking loxP sequences, was subcloned into a unique AatII site in the *Fbn1* intron before exon 38. The sequences of the loxP sites and SDM-created mutations were confirmed by direct sequencing. The vector was linearized using a unique (NruI) site and electroporated into R1 ES cells. Positive clones were identified by Southern blot analysis (Figure S1B) as previously described [1]. Positive clones were injected into 129/SvEv blastocysts at ED 3.5 and transferred into pseudopregnant females. Chimeric offspring were mated to C57Bl6/J mice, and germline transmission was observed for at least three independent targeting events for each genotype. All exons encompassed by and immediately flanking the targeting vector were analyzed by sequencing of PCR-amplified genomic DNA derived from mutant animals to demonstrate the fidelity of targeting. Complete concordance of phenotype for three or two independent lines for mutations W1572C or D1545E, respectively, excluded any major off-target effect. Mice were genotyped on the basis of creation of a new AciI site (W1572C) or destruction of a BsmAI site (D1545E) in correctly targeted mice (Figure S1C). Primers used for amplification: Sense: 5'-GATCCCACCACCTGCATC-3'; Antisense: 5'-CATGTGTTCACAGAAGGACAC-3'. The loxP-flanked NeoR was removed by breeding *Fbn1*$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice with transgenic mice that ubiquitously expresses Cre-

recombinase using a EIIa-promoter, purchased through Jackson Laboratories (Bar Harbor, ME). Over 85 embryos were genotyped at ED 10.5 for *Fbn1*$^{D1545E/+}$ homozygosity.

In vivo Drug Treatment

All antibodies used to treat mice or cells were azide-free. Male mice were treated with β1 integrin activating antibody (β1aAb, Rat Clone 9EG7, BD Biosciences, special-ordered >98% pure and azide-free) or an isotype-matched control (Rat IgG2a, κ, special- ordered >98% pure and azide-free, BD Biosciences) by intraperitoneal injection at 2 mg/kg every five days for twelve weeks, beginning at one month of age. Complete blood cell counts were performed to exclude pancytopenia in β1aAb-treated animals (Figure S16A). For the TGFβ-Neutralizing trial, three-month-old male mice were treated with pan-specific TGFβ-Neutralizing antibody (Mouse Clone 1D11, catalog #MAB1835, R&D) or an isotype control (Mouse IgG1, Clone 11711, cat# MAB002, R&D) by intraperitoneal injection at 10 mg/kg every other day for twelve weeks. RDEA119 was generously provided by Craig J. Thomas, Samarjit Patnaik, and Juan J. Marugan (National Institutes of Health Chemical Genomics Center, Rockville, MD, USA). RDEA119 was reconstituted in 10% 2-hydroxypropyl-beta-cyclodextrin (Sigma-Aldrich) dissolved in PBS, and administered twice daily by oral gavage at a dose of 25 mg/kg. Treatment was initiated at 1 month of age and continued for 8 weeks. 10% 2- hydroxypropyl-beta-cyclodextrin dissolved in PBS was administered as a control. Given the absolute concordance regarding

pathology and therapeutic responses for *Fbn1*$^{D1545E/+}$ and *Fbn1*$^{W1572C/+}$ mice seen early in this study, later studies focused on *Fbn1*$^{D1545E/+}$ mice to limit the expense associated with in vivo antibody (TGFβNAb and β1aAb) and drug (RDEA119) trials.

Stiffness Scoring

A clinical stiffness score was assigned by five blinded observers. Observers were blinded to genotype and treatment status. Mice were assessed in random order. A score of 1 indicates no stiffness (i.e. identical to wild-type mice). A score 4 indicates extreme stiffness based upon prior experience with untreated SSS mice, with 2 and 3 indicating a subjective assessment of an intermediate level of stiffness. Early in the course of studies, the same mice were assessed by the same observer on a different day. This pilot demonstrated excellent intra-observer concordance. To measure stretched skin area (SSA) and total surface area (TSA), mice were anesthetized with isofluorane and the back skin was shaved and briefly treated with Nair® cream. Area measurements were performed with NIH image J software (National Institute of Health, Bethesda, MD, USA). Mice were then briefly suspended with forceps secured to the back skin by a clamp and photographed in profile in a uniform manner (Figure S4A,B). There were no differences in body weight between all experimental groups (Figure S4C).

Histology

For tissue analysis, animals were euthanized through inhalational halothane (Sigma) or anesthetized with isofluorane. Back skin was shaved and briefly treated with Nair® cream before biopsy. Fixed skin was paraffin-embedded, sectioned, and stained with a standard Masson's trichrome stain. Dermal and subcutaneous fat thickness was measured using high-powered fields as described previously [2]. Immunofluorescent staining was performed on frozen sections as previously described [3]. Active $\alpha v\beta 3$ was detected using the WOW-1 antibody (a gift from Dr. Sanford Shattil [9]) and an anti-mouse Alexa Fluor-594 F(ab')2 fragment secondary (Invitrogen cat# A11020). Other antibodies used include Anti-CD45 antibody (BD, cat#550539), anti-Siglec H (ebiosciences cat# 14-0333-81) and antibodies to LAP1 (cat#141402, BioLegend), LAP2 (cat# LS-C137100, Lifespan BioSciences) active TGFβ1 (Clone LC(1-30), a gift from Kathleen Flanders), and total TGFβ2 (cat# ab66045, abcam). With the exception of WOW-1, all other antibodies were conjugated via an amine-based Alexa Fluor antibody labeling kit (Invitrogen, cat# A-20181, A20187, A-20185, A-20186).

Electron microscopy

Electron microscopy (EM) was performed as previously described [4].

Enzyme-linked immunosorbent assay

Mouse sera was collected and enzyme-linked immunosorbent assays (ELISAs) were performed using the Mouse Anti-Nuclear Antigens and Mouse Anti-

Scl70kits (cat#5210 and 6110, AlphaDiagnostic) according to the manufacturer's instructions.

Cell Culture

Primary human dermal fibroblasts (HDFs) were derived from skin biopsies from 5 patients with active diffuse systemic sclerosis and 6 healthy controls. Biopsies were taken from the forearm and cultured as previously described [6]. All experiments were performed in cell lines at low (<5) passage. Primary mouse embryonic fibroblasts (MEFs) were derived from E13.5 embryos as described previously [7]. Murine plasmacytoid dendritic cells (pDCs) were isolated from the spleens of wild-type C57Bl6/J mice using the Plasmacytoid Dendritic Cell Isolation Kit II (cat#130-092-786, Miltenyi Biotec) and a midiMACS™ Separator (cat#130-042-302, Miltenyi Biotec) according to the manufacturer's instructions. The pDC-containing cell suspensions routinely had greater than 95% purity, as detected by flow cytometry. For MEF/pDC co-culture experiments, MEFs were cultured to complete confluency in culture medium containing RPMI-1640, streptomycin 100 µg/ml, penicillin 100 U/ml, 2 mM L-glutamine (Gibco®) and 10% heat-inactivated fetal calf serum. 72 hours post-confluence, 5×10^6 murine splenic pDCs were plated onto MEF monolayers. After 72 hours of co-culture, both adherent and non-adherent cellular fractions were harvested, counted, and analyzed by flow cytometry.

Flow Cytometric Analysis

Mouse skin was digested for flow cytometric analysis as previously described [5]. On average, 4 x10^6 cells were obtained from a 1 x 2 cm^2 piece of skin for wild-type mice, and 8 x10^6 cells were obtained from either SSS mouse model. Murine Fc receptors were blocked using Abs against mouse CD16/32 antigens (cat# 553141, BD Biosciences). Murine plasmacytoid dendritic cells were isolated as previously reported [8]. All isolated cells (including murine dermal cells, cultured MEFs, splenic murine pDCs, or human dermal fibroblasts) were stained and fixed using the BD Cytofix/Cytoperm™ system (cat# 554722, BD Biosciences). Data were acquired using CellQuest-Pro software on a FACSCalibur flow cytometer or BD FACSuite™ software on a FACSVerse flow cytometer (BD Biosciences, San Jose, CA, USA). Data were analyzed and all flow cytometry plots were contour plots (with outliers) that were generated with FlowJo® software (TreeStar). For histograms, FlowJo software divides all events into 256 “bins,” which are numerical ranges for the parameter on the x-axis. The percent of maximum (y-axis) is the number of cells in each bin divided by the number of cells in the bin that contains the largest number of cells. Gating for live cells was based on staining with the LIVE/DEAD® Fixable Dead Cell Stain Kit (Invitrogen, cat# L34955). All staining was performed with fluorophore-conjugated primary and isotype control antibodies. All antibodies were either purchased as fluorochrome conjugates or conjugated via amine-based Alexa Fluor antibody labeling kits (cat# A-20181, A20187, A-20185, A-20186, Invitrogen). Mouse and human active $\alpha v\beta 3$ was detected fluorophore-conjugated WOW-1 antibody (a gift from Dr. Sanford Shattil). 10 mM Ethylenediaminetetraacetic acid (EDTA) and 2 mM

$MnCl_2$ were used as negative and positive controls for αvβ3 activation in flow cytometry experiments (Figure S16B) [9]. Integrin αvβ5, a subtype known to react with the WOW-1 antibody [9], was monitored in mouse and human cells with a specific antibody (cat#LS-C36943, Lifespan Biosciences). Other antibodies used on mouse cells were: integrin β1 (Clone eBioHMb1-1, cat#17-0291-80, eBiosciences), integrin β3 (Clone 2C9.G3, cat#12-0611, eBiosciences), integrin α5 (cat#11-0493-83, eBiosciences), integrin β6 (cat#LS-C152915, Lifespan BioSciences), integrin β8 (Clone H-160, cat# sc-25714, Santa Cruz Biotechnology), and pERK1/2 (cat#4370, Cell Signaling). Antibodies used for immunologic characterization of mouse cells from from ebiosciences include IL-13 (cat#53-7133-82) and IL-22 (cat#12-7221-82); from BD biosciences include: Ly6C (cat#560593), CD11b (cat#562127), CD4 (cat#560783), CD8 (cat#560469), CD19 (cat#550992), CD138 (cat#553714), IL-9 (cat#561492), IL-17 (cat#560522), IL-4 (cat#557739), IL-6 (cat#561376), IFN-γ (cat#560660), Foxp3 (cat#560047), and B220 (cat#561226); and from Biolegend® CD3 (cat# 100227), Siglec H (cat#129611). The antibody for IFN-α was from PBL interferon source (cat#22100-3). The antibody for CD317 was from eBiosciences (cat#46-3172-82). Antibodies used with human fibroblasts were: integrin β1 (Clone MAR4, cat#557332, BD biosciences) and integrin β3 (Clone VI-PL2, cat# 17-0619-42, eBiosciences).

***In vitro* TGFβ1-stimulation of Human Dermal Fibroblasts**

All cells were counted at splitting and all treatments were performed at 70% confluency. Cells were serum starved 48 hours prior to stimulation with 2 ng/mL recombinant TGFβ1 (cat#240-B-010, R&D). When TGFβ1 or vehicle was added, cell culture dishes were immediately rocked on the same rocker three times at 5% CO2, 37° to control for mechanical MAPK activation. Before lysate harvest, cells were washed with pre-warmed (42°) 1XPBS (Gibco®). All antibody treatments of human fibroblasts were added during starvation 48 hours before TGFβ1 stimulation while inhibitors SD208 (1μM) and UO126 (10 μM) (cat#s 616456 and 662005, EMD Millipore) were added 6 hours prior to stimulation. Antibodies used *in vitro* were mouse IgG1 (0.2 mg/mL, Clone P3.6.2.8.1, cat#16-4714-81, eBiosciences), IgG2a (0.2 mg/mL, Clone eBM2a, cat#16-4724, eBiosciences), αvβ3-blocking (30 μg/mL, Clone LM609, cat#MAB1976Z, Millipore), β1-activating (7 μg/mL, Clone TS2/16, cat#14-0299, eBiosciences), and β1-blocking (0.2 mg/mL, Clone P4C10, cat#MAB1987Z, Millipore) antibodies.

Western Blotting

Before lysate harvest, cells were washed with pre-warmed (42°) 1XPBS (Gibco®). Total protein was isolated from cells with ice-cold RIPA buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) with phosphatase and protease inhibitors (cat#04906837001 and cat#11836170001, Roche). Western blotting was performed using the Bio-Rad and LiCor Odyssey detection systems as previously described [6]. The relative

intensities were measured using LiCor Odyssey software. The following antibodies were used: phosphorylated and total ERK (Clone D13.14.4E, cat #4370 and Clone 3A7, cat #9107, Cell Signaling), vinculin (Clone hVIN-1, cat# V9131, Sigma), and phosphorylated and total SMAD3 (cat# 1880-1 and 1735-1, Epitomics).

RNA isolation and qPCR

Total RNA was isolated from cultured cells or tissue using Trizol (Invitrogen) according to the manufacturer's protocol. Quantitative PCR for miR-29a and 18S rRNA was performed using pre-designed Taqman primers and probes (ABI) according to manufacturer's instructions. Relative quantification for each transcript was obtained by normalizing against 18S transcript abundance according to the formula $2^{-Ct}/2^{-Ct(18S)}$.

Statistics and Graphs

All quantitative data are shown as standard boxplots produced in R statistical software. The upper and lower margins of the box define the 75^{th} and 25^{th} percentiles, respectively; the internal line defines the median, and the whiskers define the range. Statistical analysis was done using two-tailed t-test assuming equal variance between the compared groups (* $p<0.05$, ** $p<0.01$, † $p<0.001$, ‡ $p<0.0001$). Values outside of the interquartile range (IQR) are shown as open circles (R software-default), but were not excluded from or treated differently in statistical analyses.

References for Supplementary Methods

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